Commentary

Colonic mucus and ulcerative colitis

Mucus is fascinating stuff. Stringy yet slippery, permeable but tough. These properties also make it difficult to solubilise and give it a notable tendency to clog chromatographic columns and stay at the origin of electrophoretic gels. As a consequence, much of the literature has been descriptive rather than molecular. This is changing rapidly. Nine genes have so far been identified which code for the protein cores of the mucins.[1] These have multiple tandem repeats which exhibit genetic polymorphisms, raising the possibility that they may account for the genetic components of diseases which affect mucosae. They have been shown to have tissue specificity, with MUC2 being the major secreted mucin in the normal colon[2] but with a change to MUC5AC in colon cancer.[3] So far, no association between MUC gene products and benign mucosal disease has been demonstrated. It is the sugar coating, however, rather than the protein core which gives the mucus its remarkable properties and which accounts for about 60–80% of the mass of mucins. The structure of the mucin sugar (oligosaccharide) chains and the nature of the genes which determine them is proving an even harder problem to crack.

Mucin oligosaccharide chains are almost entirely initiated by O-linkage of N-acetylgalactosamine to serine or threonine in the protein core, in contrast to the oligosaccharides of most other glycoproteins which are more commonly initiated by N-acetylglucosamine, N-linked to asparagine. The O-linked structures have blood group antigenicity which immediately raises the possibility, or even probability, of genetic variation. They are also highly complex, with potential variations in their sequence, linkage, branching, and substitution by ester sulphation or O-acetylation, giving them a many-fold greater structural diversity than a similarly sized peptide.

In intestinal mucosal diseases, such as ulcerative colitis or Crohn's disease, the hypothesis that there might be an underlying genetic abnormality in mucus seems particularly attractive,[4] and there is direct evidence that the mucus layer is structurally altered, thin in ulcerative colitis and thick in Crohn's disease.[5] There is already evidence that the oligosaccharide chains in inflammatory bowel disease tend to be about half the normal length (of seven to 10 residues) and show increased expression of oncofetal carbohydrate antigens that are also found in colonic polyps and cancer,[6] and also demonstrate aberrant expression of blood group antigens.[7] These changes seem likely to be the result of alterations in the expression of specific glycosyl- and sulphotransferases. Most of the relevant enzymes have yet to be sequenced and cloned, and as 12 different N-acetylgalactosamine transferases responsible for the initiation of O-linked mucin oligosaccharide chains have already been identified, the identification and sequencing of all the relevant enzymes is going to be a formidable task. Colonic mucins are most readily distinguished from mucins elsewhere in the intestine by their high degree of sulphation but, whereas the structure of the neutral mucin

oligosaccharides in the normal human colon is largely known,[8] that of the normal sulphated oligosaccharides has yet to be determined. This is particularly important with reference to inflammatory bowel disease where reduced sulphation of mucins has been demonstrated in Europeans[9] but not Southern Asians[10] with ulcerative colitis. Rapid progress is being made in this area and it is likely that the sulphated oligosaccharides and the glycosyl- and sulphotransferase enzymes relevant to their synthesis will be identified within the next few years. Hopefully, this will allow us to fill in some of the sizable gaps that currently exist in our basic knowledge – for example: how is O-glycosylation regulated? To what extent is the structure of the O-linked oligosaccharide determined by the protein to which it is attached?

Meanwhile, it is still possible to increase our knowledge by careful descriptive studies. Matsuo and colleagues (see page 782) remind us how much important information is lost when tissue is fixed in formalin. In beautiful histochemical studies of tissue samples fixed in Carnoy's solution, they demonstrate that there are two layers to the surface mucus gel in the normal colon: a lower layer that seems to have been secreted by the goblet cells lying directly beneath it and which seems to be almost devoid of bacteria, and possibly attached to the underlying mucosa; and an upper laminated layer which contains a mixture of mucins, cell debris and bacteria, and which presumably represents an accumulation of material that has been swept down from the more proximal colon and partially degraded. The inner layer is shown to be almost entirely lacking over the surface of carcinomas and polyps. This has considerable functional relevance as it will permit close contact between the surface mucosa and intraluminal bacterial and dietary components, including lectins, which may then bind to mucosal receptors and have profound effects on mucosal proliferation.[11]

In a recent issue, Smithson and colleagues from Jewell's group have shown a further application of their ingenious technique of selective antibody development against disease related antigens.[12] They show that prior tolerisation against common colonic antigens allows them to develop antibodies that are relatively specific for the diseased colon. They have then characterised two such antibodies which they show to be highly specific for colonic mucus. Mucus is so highly glycosylated that antibodies developed against it are almost always directed against carbohydrate epitopes unless the mucin has first been deglycosylated. The two antibodies they described are no exception. One of these antibodies was able to be characterised in more detail and shown to be directed against O-acetylated or sulphated mucin, and showed reduced binding to the distal colon in ulcerative colitis. This confirms the importance of expanding our knowledge of the nature of the sulphated oligosaccharides in normal and colitic mucus. We have had to rely for far too long on the charge-based histochemical stains, usually high iron diamine and alcian blue. These give aesthetically pleasing results but in the knowledge that

there are over 20 different neutral oligosaccharide structures in colonic mucins and likely to be at least as many sulphated structures, the information given by these two-tone sepia/aquamarine portraits is frustratingly limited. Antibodies and lectins with known specificities against different oligosaccharide structures are increasingly available and immunisation with synthetic peptides based on the relatively non-glycosylated stretches of MUC proteins has also permitted production of antibodies which react with fully glycosylated mucin species.[13] Combination of these reagents with the use of mucus preserving fixatives such as Carnoy's may give us useful information about the variations in synthesis and degradation of the mucus layer(s) in colonic disease.

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